

Selective Interactions among the Multiple Connexin Proteins Expressed in the Vertebrate Lens: The Second Extracellular Domain Is a Determinant of Compatibility between Connexins

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Abstract. Gap junctions are collections of intercellular channels composed of structural proteins called connexins (Cx). We have examined the functional interactions of the three rodent connexins present in the lens, Cx43, Cx46, and Cx50, by expressing them in paired *Xenopus* oocytes. Homotypic channels containing Cx43, Cx46, or Cx50 all developed high conductance. Heterotypic channels composed of Cx46 paired with either Cx43 or Cx50 were also well coupled, whereas Cx50 did not form functional channels with Cx43. We also examined the functional response of homotypic and heterotypic channels to transjunctional voltage and cytoplasmic acidification. We show that all lens connexins exhibited sensitivity to cytoplasmic acidification as well as to voltage, and that voltage-dependent closure of heterotypic channels for a given connexin was dramatically influenced by its partner

connexins in the adjacent cell. Based on the observation that Cx43 can discriminate between Cx46 and Cx50, we investigated the molecular determinants that specify compatibility by constructing chimeric connexins from portions of Cx46 and Cx50 and testing them for their ability to form channels with Cx43. When the second extracellular (E2) domain in Cx46 was replaced with the E2 of Cx50, the resulting chimera could no longer form heterotypic channels with Cx43. A reciprocal chimera, where the E2 of Cx46 was inserted into Cx50, acquired the ability to functionally interact with Cx43. Together, these results demonstrate that formation of intercellular channels is a selective process dependent on the identity of the connexins expressed in adjacent cells, and that the second extracellular domain is a determinant of heterotypic compatibility between connexins.

ADJACENT cells can directly share ions and small metabolites through arrays of intercellular channels present in the morphological structure known as the gap junction (Revel and Karnovsky, 1967). The structural proteins comprising these channels, the connexins (Cx)¹ are a multigene family of which 12 members have been identified and cloned in rodents (Paul, 1986; Beyer et al., 1987; Zhang and Nicholson, 1989; Hoh et al., 1991; Paul et al., 1991; Haefliger et al., 1992; Hennemann et al., 1992a, 1992b; White et al., 1992). The topology of connexin proteins consists of four transmembrane domains linked by one cytoplasmic and two extracellular loops, with cytoplasmic amino and carboxy termini (Zimmer et al., 1987; Goodenough et al., 1988; Milks et al., 1988; Yancey et al., 1989). The trans-

membrane domains and extracellular loops are highly conserved among different connexins, whereas the cytoplasmic domains are often divergent (Beyer et al., 1990; Haefliger et al., 1992; Kumar and Gilula, 1992).

Multiple connexins are often expressed in the same organ, or even in the same cell type (Nicholson et al., 1987; Bennett et al., 1991; Dermietzel and Spray, 1993), and it is not currently known how the diversity of the connexin family influences intercellular communication. Intercellular channels comprised of individual connexins can respond differently to a wide variety of physiological stimuli including voltage and cytoplasmic pH (reviewed in Bennett and Verselis, 1992). Multiple connexin proteins may have evolved to provide greater regulatory flexibility, and the expression of several connexins in a single cell type could allow control of both the extent and manner of intercellular communication between adjacent cells. Connexins oligomerize to form connexons, and two connexons in apposing plasma membranes become aligned to form an intercellular channel, which is defined as homotypic when the same connexin is expressed in the two coupled cells, or heterotypic when different connexins are contributed by the adjacent cells. Although previous studies have demonstrated that many con-

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1. *Abbreviations used in this paper:* A, cooperativity constant; Cx, connexin; G_j, conductance; G_{jmin}, minimum conductance; G_{jss}, steady-state conductance; MB, modified Barth's medium; V_{i-o}, inside-outside potential; V_j, transjunctional potential; V_o, voltage at which decrease in G_{jss} is half maximal.

nexins readily form heterotypic channels in functional expression assays (Swenson et al., 1989; Werner et al., 1989; Barrio et al., 1991; Hennemann et al., 1992c), recent evidence has suggested that heterotypic channel formation can be a selective process that depends on the expression of compatible connexins in adjacent cells (Bruzzone et al., 1993).

Understanding how the molecular diversity of connexin proteins contributes to organ homeostasis is possible by examining functional interactions among the multiple connexins known to be expressed in any given organ. The vertebrate lens expresses three different gap junction proteins, connexin43 (Cx43), connexin46 (Cx46), and connexin50 (Cx50). Because the lens is avascular, its homeostasis is uniquely dependent on gap junction-mediated metabolic cooperation between cells. The lens contains only two differentiated cell types, epithelial cells on the anterior surface of the lens and specialized lens fibers that constitute most of the organ's mass. New lens fibers are continuously arising from stem cells at the lens equator, resulting in the encystment of older lens fibers. It has been proposed that gap junctional intercellular communication allows the metabolically active cells on the surface of the lens to maintain precise intracellular ionic conditions for the encysted fibers, necessary to prevent precipitation of fiber proteins and cataract formation (for review see Rae, 1979). Intercellular communication occurs between epithelial cells, between fiber cells, and between the epithelium and the fibers (reviewed in Goodenough, 1992). Epithelial gap junctions contain Cx43 (Beyer et al., 1989; Musil et al., 1990), and coupling in the epithelium is sensitive to cytoplasmic acidification (Schuetze and Goodenough, 1982; Miller and Goodenough, 1986). In contrast, lens fiber junctions contain Cx46 and Cx50 (Kistler et al., 1985; Paul et al., 1991; White et al., 1992), and intercellular communication between the fibers shows variable sensitivity to cytoplasmic acidification in different species (Benedetti et al., 1974; Schuetze and Goodenough, 1982; Bassnett and Duncan, 1988; Mathias et al., 1991; Emptage et al., 1992).

We have examined how the three connexins expressed in the vertebrate lens functionally interact in paired *Xenopus* oocytes. Homotypic channels composed of Cx43, Cx46, or Cx50 were differentially regulated by transjunctional voltage and cytoplasmic acidification. The formation of heterotypic channels among lens connexins was a selective property requiring the presence of compatible connexins in the adjacent cells. Cx50 did not form heterotypic channels with Cx43, while Cx46 was compatible with both Cx43 and Cx50. In addition, heterotypic channels displayed novel responses to transjunctional voltage, not predicted by the properties of the corresponding homotypic channels. Since selective communication among multiple connexins may be a widespread regulatory phenomenon, we have begun to investigate its molecular basis. We constructed chimeric connexins composed of parts of Cx46 and Cx50, and tested them for their ability to interact with Cx43. We show that the second extracellular domain is a determining factor of compatibility among different connexins.

Materials and Methods

In Vitro Transcription and Translation

Cx43, Cx46, and Cx50 were previously subcloned (Swenson et al., 1989;

Paul et al., 1991; White et al., 1992) into the transcription vector SP64T (Krieg and Melton, 1984). Chimeric connexins were created in the same vector (see below). Constructs were linearized with restriction endonucleases, and capped mRNAs were transcribed in vitro with SP6 RNA polymerase using the mMessage mMachine (Ambion Inc., Austin, TX) according to the manufacturer's instructions. Purity and yield of transcribed mRNA were assessed using agarose gel electrophoresis and by comparing the intensity of ethidium bromide staining to a known standard (RNA ladder; GIBCO, Grand Island, NY). In vitro-synthesized connexin mRNA (50 ng) was translated in a rabbit reticulocyte lysate (New England Nuclear, Boston, MA) in the presence of 80 mM potassium acetate, 0.65 mM magnesium acetate, and 25 μ Ci of [35 S]methionine. Translation products were separated by SDS-PAGE and detected by autoradiography.

Preparation of *Xenopus* Oocytes

Oocytes were collected from *Xenopus laevis* females and processed for the paired oocyte expression assay as described (Swenson et al., 1989), except that the final concentration of calcium in the modified Barth's medium (MB) was adjusted to 2.9 mM with CaCl_2 (Ebihara and Steiner, 1993). To eliminate the possible contribution of endogenous intercellular channels to the measured conductance, manually defolliculated oocytes were injected with an antisense oligonucleotide (Bruzzone et al., 1993) corresponding to a region within the coding sequence of *Xenopus* Cx38 (Ebihara et al., 1989; 3 ng/oocyte, 5'-CTGACTGCTCGTCTGCCACAG-3'). The efficacy of the antisense treatment was tested by measuring the recruitment of *Xenopus* Cx38 into heterotypic channels with Cx43 in the presence or absence of injected oligonucleotides. After incubation for 24–72 h at 19°C, antisense-treated oocytes were then injected with 40 nl of connexin mRNA (10–100 pg). It has been our experience that while all connexin mRNAs induce conductance in a dose-dependent manner, individual connexins vary in the specific concentration of mRNA required to achieve any fixed level of conductance. In the experiments described here, the amounts of specific connexin mRNA were varied so that homotypic channels composed of all three connexins had equivalent levels of conductance. In all cases, oocytes used for the comparison of homotypic and heterotypic channel formation received the same concentration of mRNA for each individual connexin, so that levels of homotypic vs heterotypic conductance are directly comparable. After RNA injection, oocytes were stripped of their vitelline membranes and paired in homotypic (same Cx mRNA in each oocyte) or heterotypic (different Cx mRNA injected in each oocyte) configurations.

Western Blotting

Xenopus oocytes injected with connexin mRNA or H_2O were transferred to Eppendorf tubes and homogenized on ice in 5 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 1 mM EGTA containing 20 μ M PMSF and 10 μ g/ml leupeptin (lysis buffer) by passage through a 22-gauge needle. The homogenate was centrifuged at 3,000 g at 4°C for 5 min to pellet yolk granules. The supernatant was then centrifuged at 100,000 g at 4°C for 30 min, and the membrane pellet was resuspended in fresh lysis buffer. Gel electrophoresis and Western blotting were carried out as previously described (Paul et al., 1991). Western blots were probed with the anti-Cx50 monoclonal antibody 6-4-B2-C6 (Kistler et al., 1985; White et al., 1992), the anti Cx43 [252-271] antiserum (Beyer et al., 1989), or the anti-Cx46 [411-416] antiserum (Paul et al., 1991). Primary antibodies were detected with alkaline phosphatase conjugated goat anti-mouse Ig (Boehringer Mannheim, Corp., Indianapolis, IN) or goat anti-rabbit IgG (Promega Corp., Madison, WI).

Electrophysiological Measurements

Intercellular communication was directly quantitated by double voltage clamp (Spray et al., 1981a) 24–72 h after oocyte pairing. Details of the clamp apparatus and data collection have been described previously (Swenson et al., 1989). Electrodes had a resistance of 1–2 M Ω and were filled with 3 M KCl, 10 mM EGTA, and 10 mM Hepes, pH 7.4. Both cells of a pair were initially clamped at –40 mV to ensure zero transjunctional potential. To impose a transjunctional potential, one cell was depolarized 10 mV, while the other cell was held at –40 mV. Current delivered to the cell clamped at –40 mV during the voltage pulse was equal in magnitude to the junctional current, and was divided by the voltage to yield the conductance. Oocyte pairs exhibiting conductances <5 μ S were selected for analysis of voltage dependence. Transjunctional potentials of opposite polarities were generated by hyperpolarizing or depolarizing one cell in 5- or 10-mV steps, while clamping the second cell at –40 mV. Initial currents were resolved at 5–10 ms, and steady state currents were measured at 30 s. Initial and steady state conductance values were normalized to their value at ± 10

mV and plotted against the transjunctional potential. Data were fit to a Boltzmann equation of the form

$$G_{ss} = (1 - G_{min}) / [1 + \exp(A(V - V_o))] + G_{min},$$

where G_{ss} is the steady-state conductance, G_{min} is the minimum conductance, A is the cooperativity constant, and V_o is the voltage at which the decrease in G_{ss} is half maximal (Spray et al., 1981a).

Sensitivity of the junctional conductance to the inside-outside potential (V_{i-o}) was examined by simultaneously clamping both cells in a pair to a series of different holding potentials ($V_{i-o} = -100, -80, -60, -40, -20,$ and 0 mV). Cell pairs were equilibrated at a given potential for 60 s, and then 10-mV test pulses were applied to one cell in the pair for 250 ms to measure conductance. G_{ss} were normalized to the value of $V_{i-o} = -100$ mV and plotted against the V_{i-o} , and fit to a Boltzmann equation.

Sensitivity of the junctional conductance to cytoplasmic acidification was tested by perfusing the bath (3.5 ml/min, total bath vol 5 ml) with MB equilibrated with 100% CO_2 while the cell pair was clamped at -40 mV, and depolarizing pulses of 10 mV were applied to one cell for 250 ms at 30-s intervals (Swenson et al., 1989). Junctional conductance was allowed to recover by switching the perfusion medium to MB that had not been equilibrated with 100% carbon dioxide.

Construction of Chimeric Connexins

The topologies of Cx46 and Cx50 were inferred from hydropathy plots and comparison to other connexin sequences (Paul et al., 1991; White et al., 1992). The second extracellular domain (E2) in Cx50 was substituted with that of Cx46 [Cx50*46E2], the E2 domain in Cx46 was exchanged with that of Cx50 [Cx46*50E2], and the first extracellular domain (E1) of Cx46 was replaced with that of Cx50 [Cx46*50E1] (see Fig. 5). Corresponding domains from different connexins were exchanged using PCR amplification.

To generate Cx50*46E2, two primers were synthesized to amplify the DNA encoding the E2 of Cx46 (see below). The sense primer corresponded to the first seven codons, and the antisense primer corresponded to the last six codons of the domain to be exchanged plus the next two codons in Cx46. The nucleotide sequences of the fifth and sixth codons at the end of E2 were modified to generate an *EagI* restriction site (AGG CCC changed to CGG CCG) without changing the predicted amino acid sequence (ArgPro). A second set of primers was designed to amplify Cx50 sequences, as well as the transcription vector in which Cx50 was subcloned, SP64T (see below). The sense primer corresponded to the last four codons of Cx50's E2 and the first four codons of the fourth transmembrane domain (M4). The codons for the conserved ArgPro sequence at the end of Cx50's E2 were also modified to generate an *EagI* restriction site without changing the predicted amino acid sequence (CGG CCT changed to CGG CCG). The antisense primer corresponded to the last seven codons of the third transmembrane domain of Cx50 (M3). The E2 domain of Cx46 (5 ng) was amplified with Vent polymerase (New England Biolabs, Beverly, MA) in a thermal cycler (GeneAmp 9600; Perkin-Elmer Cetus Instruments, Norwalk, CT) in 25 cycles of $94^\circ C$ for 30 s, $58^\circ C$ for 30 s, and $72^\circ C$ for 30 s. Cx50 in the SP64T vector (5 ng) was amplified with 25 cycles of $94^\circ C$ for 30 s, $58^\circ C$ for 30 s and $72^\circ C$ for 5 min. The PCR amplified products were phosphorylated, digested with *EagI*, separated by agarose gel electrophoresis, and purified (Gelase; Epicenter Technologies Corp., Madison, WI). Purified products were ligated together to generate a chimera where the E2 of Cx50 (amino acids 174–206) was replaced with the corresponding E2 of Cx46 (amino acids 171–203).

To create Cx46*50E2, a similar strategy was devised, except that the nucleotide sequences of the primers (see below) altered the codons at the end of Cx46 and Cx50's E2s to generate an *XhoI* restriction site without changing the predicted amino acid sequence (ValSerArg; A TCC CG or C TCC AG changed to C TCG AG). The E2 domain of Cx50, and Cx46 in the SP64T vector were amplified as described for Cx50*46E2. The PCR-amplified products were phosphorylated, digested with *XhoI*, separated by agarose gel electrophoresis, and purified (Gelase; Epicenter Technologies Corp.). Purified products were ligated together to generate a chimera where the E2 of Cx46 (amino acids 171–203) was replaced with the corresponding E2 of Cx50 (amino acids 174–206).

To construct Cx46*50E1, two oligonucleotides that corresponded to adjacent regions in the E1 domain of Cx46 were synthesized. The sense primer contained the nucleotides encoding amino acids 61–72 of Cx46, with codon 68 altered to correspond to amino acid 68 of Cx50 (Arg to Glu, CGT changed to GAA). The antisense primer coincided with amino acids 49–60 of Cx46, with codon 53 modified to correspond to amino acid 53 of Cx50 (Thr to Val, ACC changed to GTC). Cx46 in the SP64T vector was amplified with 25 cycles of $94^\circ C$ for 30 s, $58^\circ C$ for 30 s, and $72^\circ C$ for 5 min.

The linear PCR amplified product was phosphorylated, gel purified (Gelase; Epicenter Technologies Corp.), and ligated to itself to generate a chimera where the E1 of Cx46 now contained the two divergent amino acids present in the corresponding E1 of Cx50 (amino acids 53 and 68).

Chimeric connexin constructs were sequenced to ensure that PCR amplification did not introduce random mutations (Biopolymer Facilities, Harvard Medical School, Boston, MA). The sequences of PCR primers are given below; codons with altered nucleotides are underlined.

Chimera Cx50*46E2

Cx46E2 Sense 5'-GCGGGCCAGTACTTTCTATAT-3'

Cx46E2 Antisense 5'-CTCCGTCGGCCGGGAGATGAAGCA-3'

Cx50M4 Sense 5'-GTATCCCGCCGACTGAGAAGACC-3'

Cx50M3 Antisense 5'-GATGAAGCCCACTCAAAGAG-3'

Chimera Cx46*50E2

Cx50E2 Sense 5'-GTGGGCCATTACTTCTGTATGG-3'

Cx50E2 Antisense 5'-GTAGGCCCTCGAGACAAAGCAGTCTACCAC-3'

Cx46M4 Sense 5'-CTTCATCTCGAGGCCACGGAGAAGAC-3'

Cx46M3 Antisense 5'-GATGAACCCCACTTCGAAGAG-3'

Chimera Cx46*50E1

Cx46E1 Sense 5'-TGTGAGAACGCTGCTACGACGAAGCCTTCCCC-ATC-3'

Cx46E1 Antisense 5'-GCCTGGCTGCTGTGTGTTGCAGACGAAGTCCG-ACTGC-3'

Results

Expression of Lens Connexin mRNAs in *Xenopus* Oocytes

The efficiency of translating exogenously supplied connexin mRNA in *Xenopus* oocytes was examined by assaying cells that received Cx43, Cx46, or Cx50 mRNA with specific antibodies. Membrane fractions prepared from oocytes injected with synthetic connexin mRNA or water were used for polyacrylamide gel electrophoresis and Western blotting (Fig. 1). Oocytes injected with Cx43 mRNA synthesized protein recognized by an antipeptide polyclonal antibody directed against residues 252–271 of Cx43 (Beyer et al., 1989). Cx46-injected cells produced an $\approx 46,000$ - M_r protein detected by the antipeptide antisera raised against amino acids 411–416 in Cx46 (Paul et al., 1991). Oocytes that received Cx50 mRNA synthesized an $\approx 63,000$ - M_r protein identified

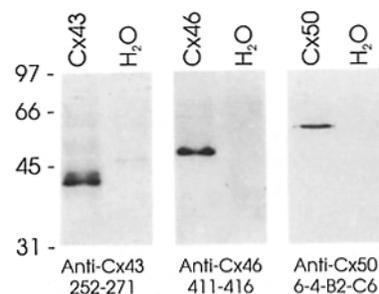


Figure 1. Lens connexins are expressed in *Xenopus* oocytes injected with RNA. Membranes were isolated from oocytes injected with connexin mRNA or H_2O , and the proteins were separated by SDS-PAGE and transferred to nitrocellulose. The Western blots were probed with the anti-Cx43 peptide 252–271 antibody, the anti-Cx46 peptide 411–416 antibody, or the anti-Cx50 monoclonal antibody 6-4-B2-C6. All three antibodies recognized a single major protein of the correct apparent molecular weight in oocytes that received the respective connexin mRNA. No major proteins were recognized in H_2O -injected oocytes. Molecular weight markers are indicated in kilodaltons.

Table I. An Antisense Cx38 Oligonucleotide Eliminates Endogenous Contributions to G_j

Oocyte injection (cell1/cell2)	G_j^*	Number of pairs
	μS	
Cx43/H ₂ O	5.34 ± 0.43	5
Cx46/H ₂ O	<0.05	12
Cx50/H ₂ O	<0.05	11
Cx43/antisense Cx38	0.09 ± 0.03	23

* Values are mean ± SE.

by the anti-Cx50 monoclonal antibody 6-4-B2-C6 (Kistler et al., 1985; White et al., 1992). None of the antibodies detected any major proteins in the control membrane fractions prepared from water-injected oocytes.

The Inhibition of Endogenous *Xenopus* Connexins

We have used the paired *Xenopus* oocyte system (Dahl et al., 1987; Swenson et al., 1989) to examine the possible interactions of the three connexins expressed in the vertebrate lens. It has been previously demonstrated that some connexins, including Cx43, can heterotypically interact with an endogenous *Xenopus* oocyte connexin, Cx38 (Swenson et al., 1989; Werner et al., 1989; Hennemann et al., 1992a; Bruzzone et al., 1993). To ensure that all measured conductances were the result of the exogenously supplied connexins, we first determined whether Cx46 or Cx50 could interact with Cx38 to form heterotypic channels, and then suppressed recruitment of the endogenous connexin by pretreating oocytes with an antisense oligonucleotide (Bruzzone et al., 1993). Neither Cx46- nor Cx50-injected oocytes developed detectable conductances when paired with water-injected cells, and injection of an antisense Cx38 oligonucleotide significantly reduced recruitment of Cx38 by Cx43 (>98%, Table I). All further experiments were done with antisense-treated oocytes.

The Formation of Intercellular Channels is a Selective Process

Functional interactions among lens connexins were determined by pairing oocytes injected with Cx43, Cx46, or Cx50 mRNA homotypically (the same mRNA injected in each cell) or heterotypically (different mRNAs injected in the two paired cells). All three connexins efficiently made homotypic channels, while the formation of heterotypic channels was a selective process dependent on which connexin was expressed in the adjacent cell. Table II provides a summary of the functional relationships among the lens connexins. The values presented in Table II are limited to only those low conductance pairs that were used for analysis of voltage dependence (see below), but are representative of many experiments over a wide range of conductances. Cx46 made heterotypic channels with both Cx43 and Cx50, but Cx50 did not form functional heterotypic channels when paired with Cx43 (compare with Cx43/antisense Cx38 in Table I). In the lens, Cx43-expressing epithelial cells are adjacent to fiber cells containing both Cx46 and Cx50. Because functional interactions among these three connexins were selective, we tested whether a cell expressing both Cx46 and Cx50 could still be coupled to a Cx43-containing cell. Oocytes injected

Table II. Heterotypic Channel Formation Is a Selective Process

Oocyte injection (cell1/cell2)	G_j^*	Number of pairs†
	μS	
Homotypic		
Cx43/Cx43	4.10 ± 0.91	10
Cx46/Cx46	2.71 ± 0.34	4
Cx50/Cx50	4.49 ± 1.69	5
Heterotypic		
Cx43/Cx46	3.59 ± 0.54	6
Cx46/Cx50	2.86 ± 1.07	7
Cx43/Cx50	0.06 ± 0.03	13
Cx43/Cx46 + Cx50	3.09 ± 1.29	5

* Values are mean ± SE.

† Only data from the low conductance pairs used to determine voltage sensitivity are included.

with both Cx46 and Cx50 mRNAs in a 1:1 ratio were paired with Cx43 injected cells and were assayed for junctional conductance. These pairs were also well coupled, demonstrating that the incompatibility of Cx50 with Cx43 did not interfere with the formation of heterotypic channels between Cx46 and Cx43.

Homotypic Intercellular Channels Display Unique Voltage Properties

Because intercellular channels composed of the three lens connexins may be uniquely regulated by physiological stimuli, we examined the responses of homotypic Cx43, Cx46, and Cx50 intercellular channels to applied transjunctional voltages. Only oocyte pairs that displayed conductances in a range of 1–5 μS were selected for voltage analysis (Table II) to avoid possible overestimation of the actual transjunctional voltage measurement because of differences in access resistance (Jongsma et al., 1991). Fig. 2 shows typical transjunctional currents and plots of normalized conductance (G_j) vs transjunctional potential (V_j) for homotypic oocyte pairs.

The junctional current in Cx43 homotypic pairs decayed slowly over time at relatively large potentials (>40 mV, Fig. 2 A). This decay was slightly asymmetrical, with positive V_j s inducing a greater rate of decline over the duration of the voltage step. This asymmetry was quantified by plotting the normalized steady-state conductance (filled circles, $n = 6$), and fitting the data to a Boltzmann equation (Spray et al., 1981a; Table III). The asymmetry was reflected in the value of the transjunctional potential at which the transition between maximum and minimum conductance was halfway (V_o). For positive V_j s, Cx43 pairs had a V_o of 55 mV, and for negative V_j s, they displayed a V_o of 59 mV. The asymmetry was even more apparent in the value of the minimum conductance ($G_{j,min}$), which was 18% of the initial value for large positive potentials and 40% of the initial value for large negative potentials. Initial conductance (open squares) measured 5–10 ms after the voltage step was stably established did not change over the range of V_j s tested (± 95 mV).

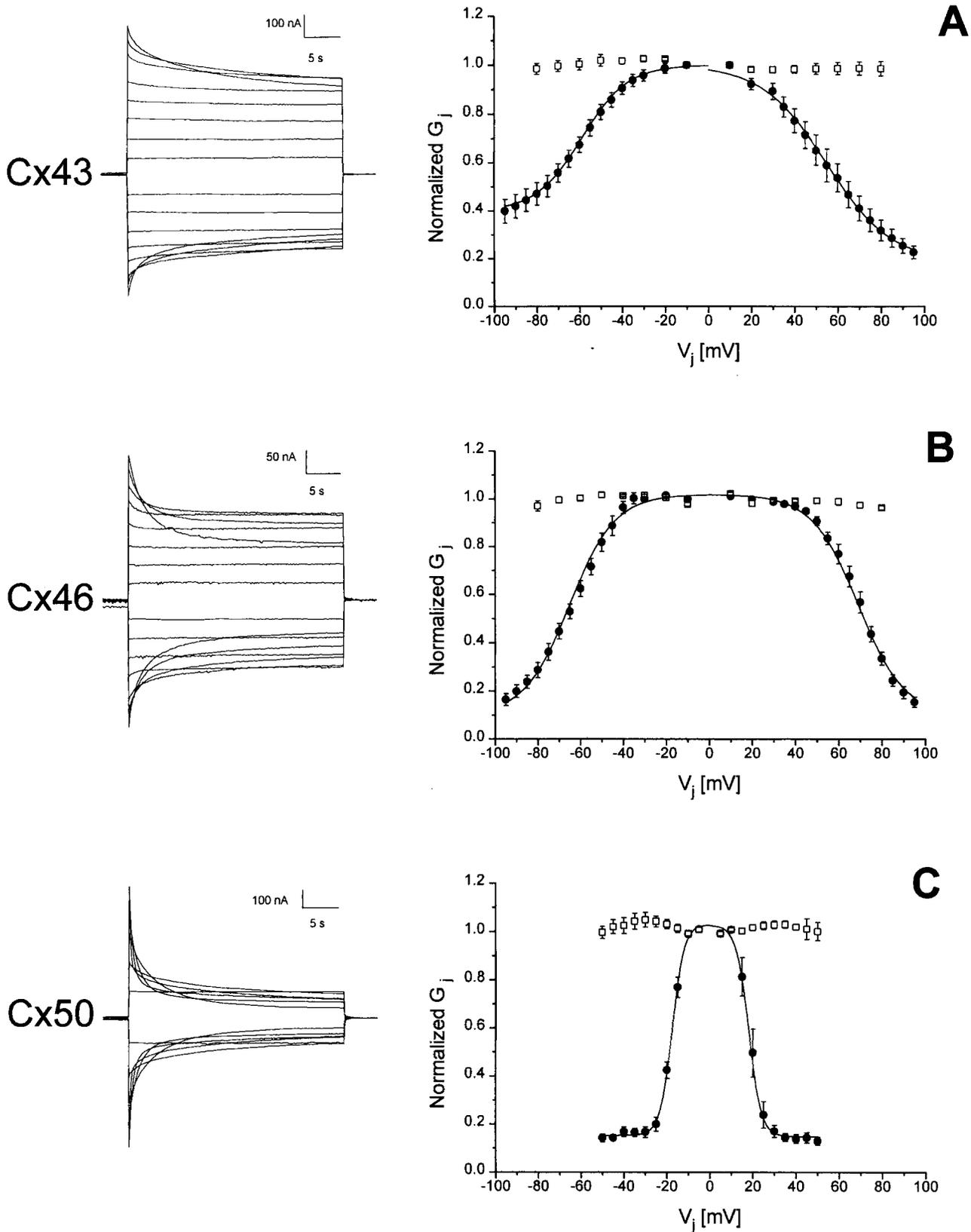


Figure 2. Homotypic channels composed of lens connexins exhibit unique voltage sensitivities. Oocytes were treated with an antisense *Xenopus* Cx38 oligonucleotide, injected with Cx43, Cx46, or Cx50 mRNA, and homotypically paired. Cells were initially voltage clamped at -40 mV, conductance was measured after the imposition of transjunctional potentials ranging from 10 to 95 mV, and normalized to the initial conductance values at transjunctional potentials of ± 10 mV. Typical junctional currents (*left*) and plots of mean initial (\square) and steady state (\bullet) conductance vs transjunctional voltage (*right*) are presented for Cx43 (*A*), Cx46 (*B*), and Cx50 (*C*). Smooth lines are computer-fitted Boltzmann equations whose parameters are given in Table III.

Table III. Boltzmann Parameters of Connexins Expressed in *Xenopus Oocytes**

Cx (condition)‡	A	n	V _o	G _{jmin}
			mV	
Homotypic (this study)				
Cx43 (+V _j)	0.07	1.8	55	0.18
Cx43 (-V _j)	0.09	2.2	59	0.40
Cx46	0.09	2.2	67	0.10
Cx50	0.34	8.5	18	0.14
Heterotypic§ (this study)				
Cx46/Cx43 (+V _j)	0.08	2.0	83	0.49
Cx46/Cx43 (-V _j)	0.13	3.3	38	0.05
Cx46/Cx50 (+V _j)	0.17	4.3	27	0.12
Cx46/Cx50 (-V _j)	0.17	4.3	28	0.18
Cx46/Cx50 (-V _j , fast)	0.14	3.5	64	0.76
Homotypic (previous studies)				
Cx26 (+V _j)	0.14	3.5	82	0.17
Cx26 (-V _j)	0.14	3.5	89	0.36
Cx30.3	0.08	2	72	0.23
Cx32	0.09	2.2	55	0.19
Cx37 (fast)	0.09	2	60	0.40
Cx37 (S ₁)	0.44	11	16	S ₂
Cx37 (S ₂)	0.09	2	40	0.12
Cx40	0.32	8	35	0.19

* Data were fit to a Boltzmann equation of the form $G_{jss} = (1 - G_{jmin}) / [1 + \exp(A[V - V_o])] + G_{jmin}$, where the cooperativity constant A can also be expressed as the gating charge n, where $n = A(kT/q)$ k = Boltzmann constant, T = absolute temperature, and q = elementary charge.

‡ ± V_j refers to the polarity of the transjunctional potential. If not indicated, response was symmetrical. "Fast" refers to instantaneous changes in G_j. S₁ and S₂ are two superimposed slow voltage responses (see Willecke et al., 1991).

§ In both cases, the Cx46 cell was held at a constant voltage, while the other cell (either Cx43 or Cx50) was hyperpolarized or depolarized. Thus, positive potentials indicate that the Cx43 or Cx50 injected cell is relatively positive, while negative potentials indicate that the Cx46 cell is relatively positive.

|| Cx26 and Cx32 data are from Barrio et al. (1991); Cx30.3, Cx37, and Cx40 data are from Henemann et al. (1992a, 1992c).

Oocyte pairs expressing Cx46 homotypic channels also displayed voltage sensitivity at large transjunctional potentials (Fig. 2B). Unlike Cx43, the junctional currents decayed symmetrically at positive and negative V_js greater than 40 mV. The normalized steady-state conductance (filled circles, n = 4) declined symmetrically with a V_o of 67 mV, and for large potentials of both polarities, the minimum conductance was 10% of the initial value. Similar to Cx43, the instantaneous conductance (open squares) of Cx46 homotypic pairs was unaffected by transjunctional voltage.

In contrast to both Cx43 and Cx46, Cx50 homotypic pairs displayed a much greater sensitivity to voltage (Fig. 2C). The junctional currents decayed rapidly at V_js >10 mV, and the normalized steady-state conductance (filled circles, n = 5) decreased symmetrically with a V_o of 18 mV. For potentials >25 mV of either polarity, the G_{jmin} was 14% of the initial conductance, and the entire transition from maximum to minimum conductance occurred between transjunctional potentials of 10–30 mV relating to a cooperativity constant (A) of 0.34, or a gating charge (n) of 8.5. Comparable to Cx43 and Cx46, the instantaneous conductance (open squares) of Cx50 homotypic pairs did not change over the range of V_js tested (±50 mV).

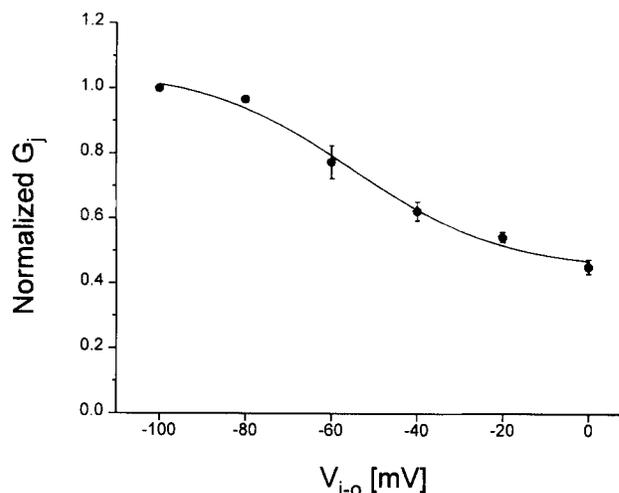


Figure 3. Cx43 exhibited a dependence on the potential difference between the cell cytoplasm and outside bath (V_{i-o}). Antisense-treated oocytes were injected with Cx43 mRNA and paired homotypically. Both cells in a pair were simultaneously clamped to a series of holding potentials (V_j = 0; V_{i-o} = -100, -80, -60, -40, -20, and 0 mV). Cell pairs were equilibrated at a given potential for 60 s, and then 10-mV test pulses were applied to one cell in the pair for 250 ms to measure conductance. G_js were normalized to the value of V_{i-o} = -100 mV, plotted against the V_{i-o}, and fit to a Boltzmann equation with the following parameters: A = 0.06, V_o = -55 mV, G_{jmin} = 0.44, and G_{jmax} = 1.06.

The unique asymmetric response of homotypic Cx43 channels was further investigated by analyzing the dependence of Cx43 on the potential difference between the cell cytoplasm and the outside bath (V_{i-o}, Barrio et al., 1991). Cx43 showed a dependence on V_{i-o}, such that more positive holding potentials resulted in a decrease in the measured conductance (Fig. 3). Fitting these data to a Boltzmann equation revealed that the predicted value of G_{jmin} for this V_{i-o} dependence (42% of G_{jmax}) was in excellent agreement with the asymmetric values of G_{jmin} observed for the Boltzmann fits of V_j dependence for homotypic Cx43 pairs (18% for +V_j, 40% for -V_j, or a net difference of 45%).

Homotypic channels composed of Cx43, Cx46, or Cx50 differed significantly in their response to imposed transjunctional voltages. By plotting the conductance vs the transjunctional potential and fitting the data to Boltzmann equations, the unique properties of these three connexins have been quantitated. Parameters previously determined for other connexins analyzed in the oocyte system have also been provided for comparison in Table III.

Heterotypic Intercellular Channels Exhibit Novel Voltage Sensitivity

To examine how the functional behavior of heterotypic channels related to the properties of homotypic channels, voltage sensitivities of heterotypic Cx46/Cx43 and Cx46/Cx50 channels were determined. Fig. 4 presents plots of normalized conductance (G_j) vs transjunctional potential (V_j) for heterotypic oocyte pairs. In both cases, the Cx46 cell was held at a constant voltage while the other cell (either Cx43 or Cx50) was hyperpolarized or depolarized. Thus, positive potentials indicate that the Cx43 or Cx50 injected cell was

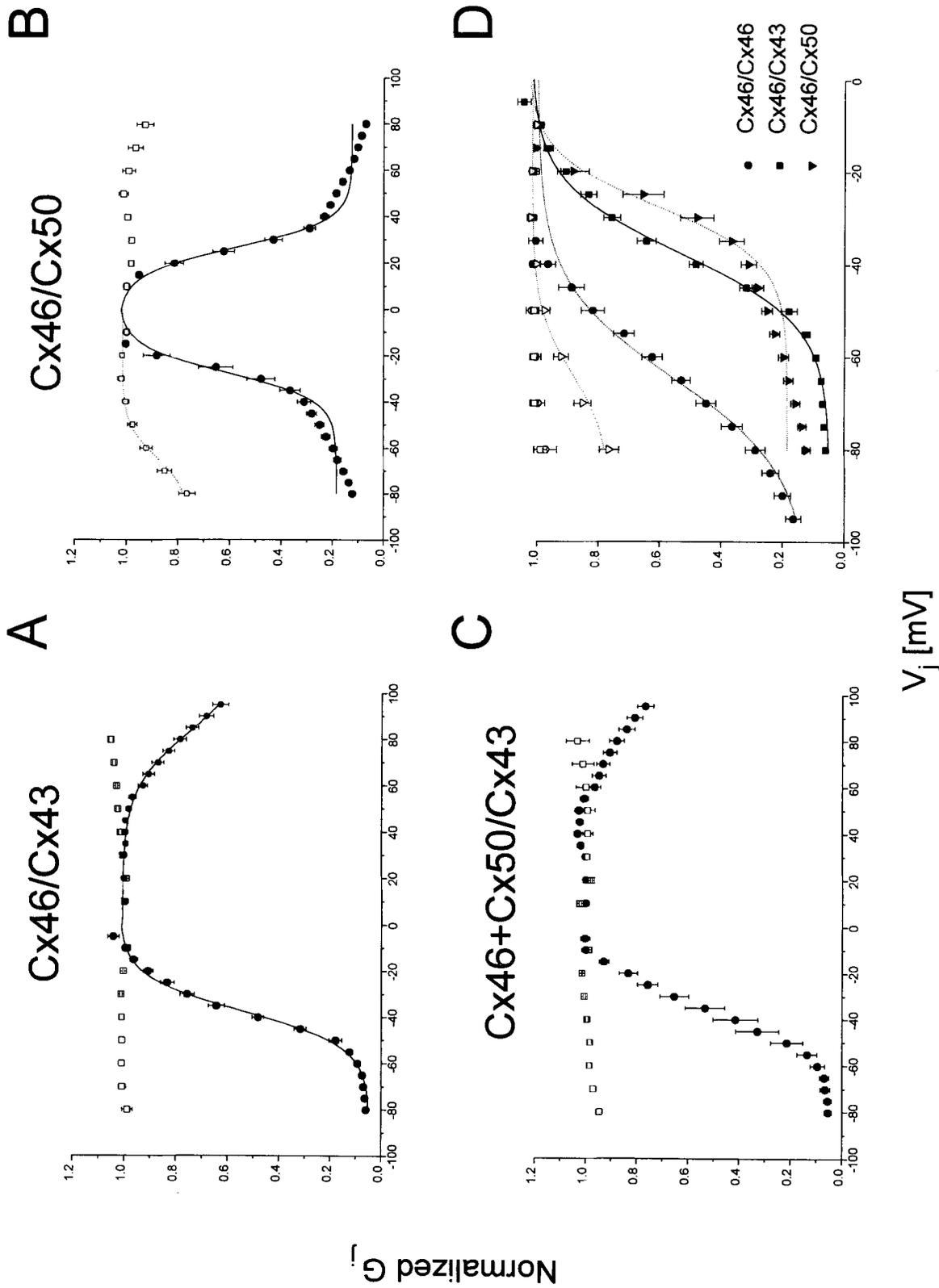


Figure 4. Heterotypic channels acquire novel voltage properties not predicted by their homotypic counterparts. Antisense-treated oocytes were heterotypically paired, and transjunctional potentials were applied as described for Fig. 2. Plots of mean initial (\square) and steady-state (\bullet) conductance vs transjunctional voltage are presented for Cx46/Cx43 (A) and Cx46/Cx50 (B) heterotypic channels. Smooth lines are computer-fitted Boltzmann equations whose parameters are given in Table III. (C) No evidence for heteromeric connexons was observed in oocytes coexpressing Cx46 and Cx50. The voltage properties of intercellular channels in Cx46+Cx50/Cx43 oocyte pairs were identical to those in Cx46/Cx43 pairs. (D) The voltage-dependent closure of Cx46 was influenced by its partner connexins. Normalized values are shown for the instantaneous (open symbols) and steady-state (filled symbols) conductance of a Cx46-injected cell when paired with a Cx46-(\bullet), Cx43-(\blacksquare), or Cx50-(\blacktriangledown) containing oocyte.

relatively positive, while negative potentials indicate that the Cx46 cell was relatively positive. It was convenient to systematically hold the Cx46 cell at a constant voltage because depolarization of Cx46-containing oocytes tends to open nonjunctional Cx46 channels (see Paul et al., 1991; Ebihara and Steiner, 1993). We have also tested hyperpolarizing or depolarizing the Cx46 cell while holding the other cell (either Cx43 or Cx50) at constant voltage, and we have seen no evidence for V_{i-o} dependence in these heterotypic pairs (not shown).

Heterotypic channels containing Cx43 and Cx46 showed highly asymmetric steady state voltage properties (Fig. 4 A, filled circles, $n = 6$). Moreover, the voltage sensitivities of both Cx43 and Cx46 in heterotypic configuration were altered compared to their values in homotypic configurations. The V_o when the Cx43-containing cell was relatively positive was 84 mV (compared to 55 mV for homotypic, Table III). In contrast, when the Cx46 cell was relatively positive, the V_o was significantly lower than that of homotypic Cx46 pairs.

Surprisingly, heterotypic pairs composed of Cx46 and Cx50 displayed a nearly symmetrical steady-state G_j vs V_j relationship (Fig. 4 B, filled circles, $n = 7$) despite large differences in the responses of homotypic Cx46 or Cx50 intercellular channels to transjunctional voltage. The cooperativity constants were identical when either the Cx46 cell or the Cx50 cell was relatively positive ($A = 0.17$, Table III), and this symmetry was also reflected in the similar values of V_o for positive and negative transjunctional potentials (27 and 28 mV). In addition, heterotypic Cx46/Cx50 pairs developed a novel instantaneous voltage dependence when the Cx46-injected cell was relatively positive (open squares).

Lens fibers express both Cx46 and Cx50, which potentially could assemble into a heteromeric connexon. We have shown that oocytes expressing both Cx46 and Cx50 were coupled to Cx43-containing cells (Table II). If heteromeric connexons are assembling in these oocytes, it is possible that these could result in intercellular channels with physiological properties different from those of the Cx46/Cx43 heterotypic channels. Fig. 4 C demonstrates that intercellular channels resulting from the coexpression of Cx50 and Cx46 in oocytes that were paired with Cx43-expressing cells had voltage properties that were indistinguishable from those of Cx46/Cx43 channels (compare with Fig. 4 A), thus providing no evidence for cooligomerization of Cx46 with Cx50.

In addition to heterotypic channel formation being a selective process, the voltage dependent closure of a given connexin was markedly influenced by the opposing connexin with which it was paired. Fig. 4 D summarizes the changes observed in the instantaneous (open symbols) and steady state (filled symbols) voltage properties for a Cx46-injected cell when paired with a Cx46-, Cx43-, or Cx50-containing oocyte. These results, combined with the changes in voltage sensitivity observed for Cx50 in homotypic vs heterotypic configurations, demonstrate that interactions between opposing connexons generate intercellular channels with novel properties.

All Rodent Lens Connexins Are pH Sensitive

The functional responses of rodent lens intercellular channels to cytoplasmic acidification were also determined by

perfusing oocyte pairs containing homotypic and heterotypic channels composed of Cx43, Cx46, and Cx50 with medium that had been equilibrated with 100% CO_2 (Swenson et al., 1989). Homotypic channels composed of either Cx43, Cx46, or Cx50 all exhibited pH sensitivity (Fig. 5 A). Conductance levels decreased $\geq 90\%$ from their initial values within 10–15 min after perfusion with carbon dioxide-equilibrated medium. This reduction in conductance was fully reversible upon switching the perfusion medium to normal buffer. Heterotypic Cx43/Cx46 and Cx46/Cx50 were also sensitive to cytoplasmic acidification in a reversible manner (Fig. 5 B).

Because both the rate and extent of pH sensitivity appeared to differ for each connexin, we examined the initial response to intracellular pH by plotting the mean decrease in conductance for homotypic Cx43, Cx46 and Cx50 oocyte pairs ($n = 3$ for each connexin) during perfusion with 100% CO_2 -equilibrated medium (Fig. 5 C). Cx43 responded as described previously (Swenson et al., 1989; Liu et al., 1993), showing an initial increase in G_j ($\sim 30\%$ in 3 min) followed by a slow decline to a value equal to 20% of the initial conductance after 10 min of perfusion. Longer times of perfusion resulted in complete closure of the Cx43 channels. Cx46 homotypic pairs became uncoupled within 5 min to a value $\geq 10\%$ of the initial conductance. This residual G_j could not be further reduced, even after perfusion with 100% CO_2 -equilibrated medium for 40 min (not shown). Cx50 exhibited a rapid and complete closure within 5 min of exposure to 100% CO_2 . Thus, all rodent lens connexins exhibited pH sensitivity when expressed in paired *Xenopus* oocytes, although the rate and degree of uncoupling differed for each connexin.

The Second Extracellular Domain may Confer Heterotypic Compatibility

We have shown that among the lens connexins, the ability to form heterotypic channels is a selective property dependent on the expression of compatible connexins in the adjacent cells. To examine the molecular basis of selective communication, we took advantage of the ability of Cx43 to discriminate between Cx46 and Cx50, and constructed chimeric connexins composed of portions of Cx46 and Cx50. The topology of connexin proteins suggests that molecules in the membranes of adjacent cells may interact through their extracellular domains. Aligning the predicted amino acid sequences of the first (E1) and second (E2) extracellular domains of Cx46 and Cx50 (Fig. 6) showed that E1 was highly conserved between these two connexins (94% identity), although there was a difference in net charge of two (E to R). The second extracellular domain was more variable, although still well conserved (76% identity), and it had a net charge difference of three (H to Q, R to Q, and S to D). To examine what role these domains may have in heterotypic recognition, the first extracellular domain in Cx46 was replaced with the E1 of Cx50 (Cx46*50E1), and the second extracellular domains were exchanged between Cx46 and Cx50 (Cx46*50E2, Cx50*46E2, Fig. 6).

To verify that these chimeric connexins were efficiently synthesized, Cx46, Cx50, Cx46*50E1, Cx46*50E2, and Cx50*46E2 mRNAs were translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine. In vitro-translated proteins were separated by polyacrylamide gel elec-

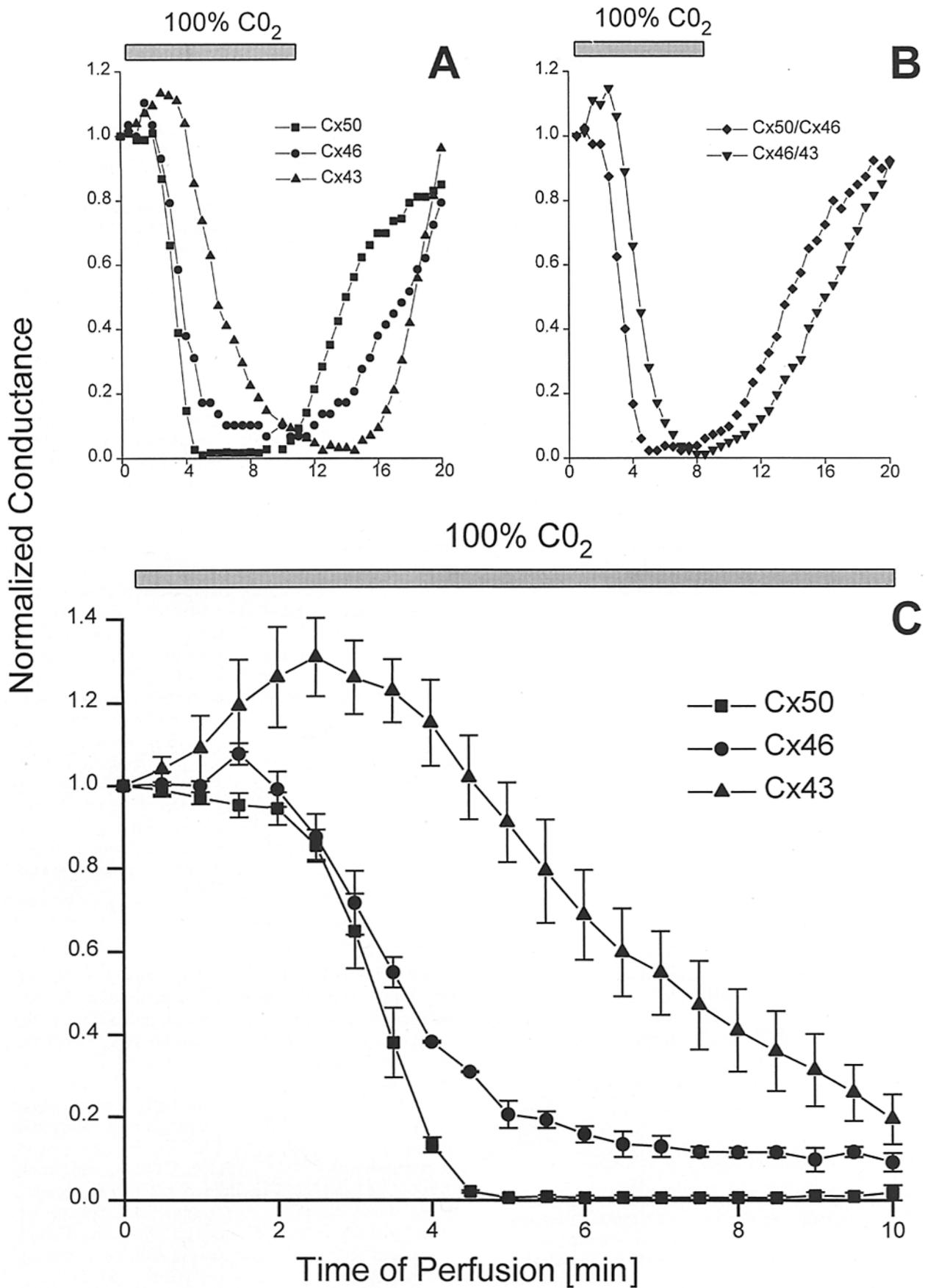


Figure 5. All rodent lens connexins are sensitive to cytoplasmic acidification. Oocyte pairs were perfused with MB equilibrated with 100% CO₂ (gray bar) after which, the perfusion medium was then switched to normal MB to allow junctional conductance to recover. For both homotypic (A) and heterotypic (B) channels composed of Cx43, Cx46, or Cx50, conductance levels decreased 90% from their initial values within 10 min. This reduction in conductance was fully reversible upon switching the perfusion medium to normal buffer. Plotting the mean decrease ($n = 3$ for each connexin) in conductance for homotypic Cx43, Cx46, and Cx50 oocyte pairs during perfusion with 100% CO₂-equilibrated medium (C) demonstrated that the rate of uncoupling differed for each connexin, with Cx50 > Cx46 >> Cx43.

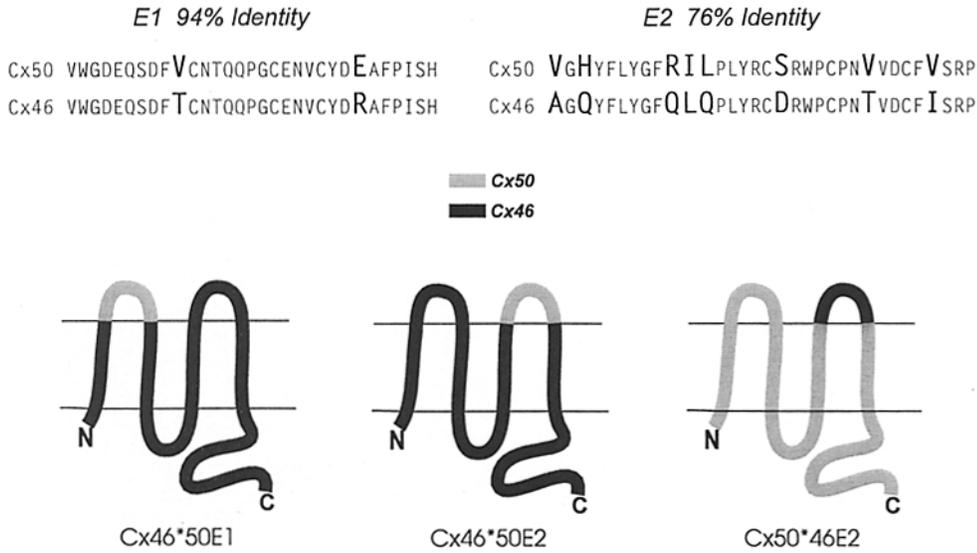


Figure 6. Schematic representation of chimeric connexins constructed by exchanging the extracellular domains of Cx46 and Cx50. Comparison of amino acid sequences of the extracellular domains of Cx46 and Cx50 demonstrated that the first extracellular domains (E1) are 94% identical and that the second extracellular domains (E2) are 76% identical. Divergent amino acids are indicated in bold type. Chimeric connexins were constructed to replace the E1 of Cx46 with that of Cx50 (Cx46*50E1), to replace the E2 in Cx46 with that of Cx50 (Cx46*50E2), and to replace the E2 in Cx50 with that of Cx46 (Cx50*46E2). Domains composed of Cx46 and Cx50 are indicated in black and gray, respectively.

trophoresis and detected by autoradiography (Fig. 7). Translation reactions that received either Cx46 or Cx50 mRNA produced predominant proteins with electrophoretic mobilities identical to previously reported values (Paul et al., 1991; White et al., 1992). The translation reactions that contained chimeric Cx46*50E1, Cx46*50E2, or Cx50*46E2 mRNA produced proteins of the same size as their respective wild-type connexins. A translation reaction receiving only H₂O synthesized no major protein products.

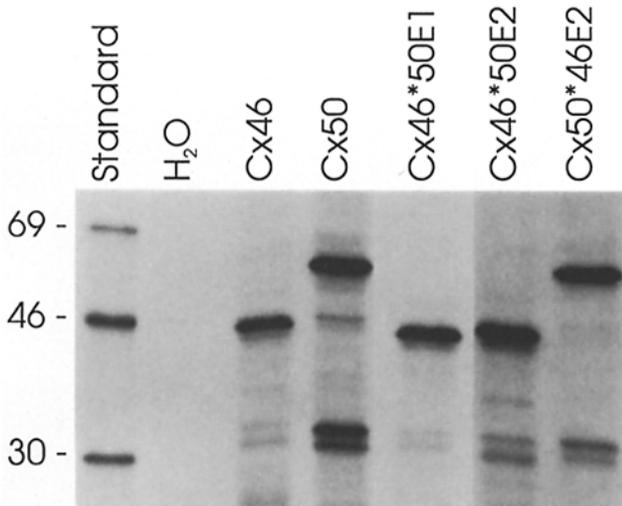


Figure 7. Chimeric connexin mRNAs are efficiently translated. Cx46, Cx50, Cx46*50E1, Cx46*50E2, and Cx50*46E2 mRNAs were translated in a reticulocyte lysate in the presence of [³⁵S]methionine, separated by SDS-PAGE, and detected by autoradiography. In vitro-synthesized chimeric connexins had electrophoretic mobilities identical to Cx46 and Cx50. Molecular mass markers are indicated in kilodaltons.

Chimeric constructs were then tested for their ability to form heterotypic channels when paired with Cx43 (Fig. 8). Heterotypic Cx43/Cx46*50E1 and Cx43/Cx50*46E2 pairs were well coupled, while Cx43/Cx46*50E2 pairs were not coupled above background levels (compare with 43/H₂O). Cx46*50E2 was also tested for its ability to make functional intercellular channels with Cx46 and Cx50. Heterotypic Cx46/Cx46*50E2 and Cx50/Cx46*50E2 pairs were also well coupled, demonstrating that this construct was able to participate in channel formation. Therefore, while the E1 domain of Cx50 did not prevent heterotypic communication between Cx46 and Cx43, the E2 domain of Cx50 inhibited interaction between Cx46 and Cx43. Moreover, the E2 domain of Cx46 conferred upon Cx50 the novel ability to form heterotypic channels with Cx43. These results demonstrate that the second extracellular domain of connexin proteins is a determinant of heterotypic compatibility.

Discussion

We have explored how the molecular diversity of the connexin family may contribute to organ homeostasis in the vertebrate lens by examining the functional interactions of the three lens connexins in paired *Xenopus* oocytes. Homotypic channels composed of either Cx43, Cx46, or Cx50 were uniquely regulated by transjunctional voltage and cytoplasmic acidification. The formation of heterotypic channels was a selective property, requiring the expression of compatible connexins in the adjacent cells. Cx50 did not form heterotypic channels with Cx43, even though Cx46 was compatible with both Cx43 and Cx50. In addition, heterotypic channels displayed novel responses to transjunctional voltage not predicted by the properties of homotypic channels. We have also investigated the molecular basis of selective communication by constructing chimeric connexins in which the extracellular domains of Cx46 and Cx50 were exchanged. These chimeras demonstrated that the second extracellular

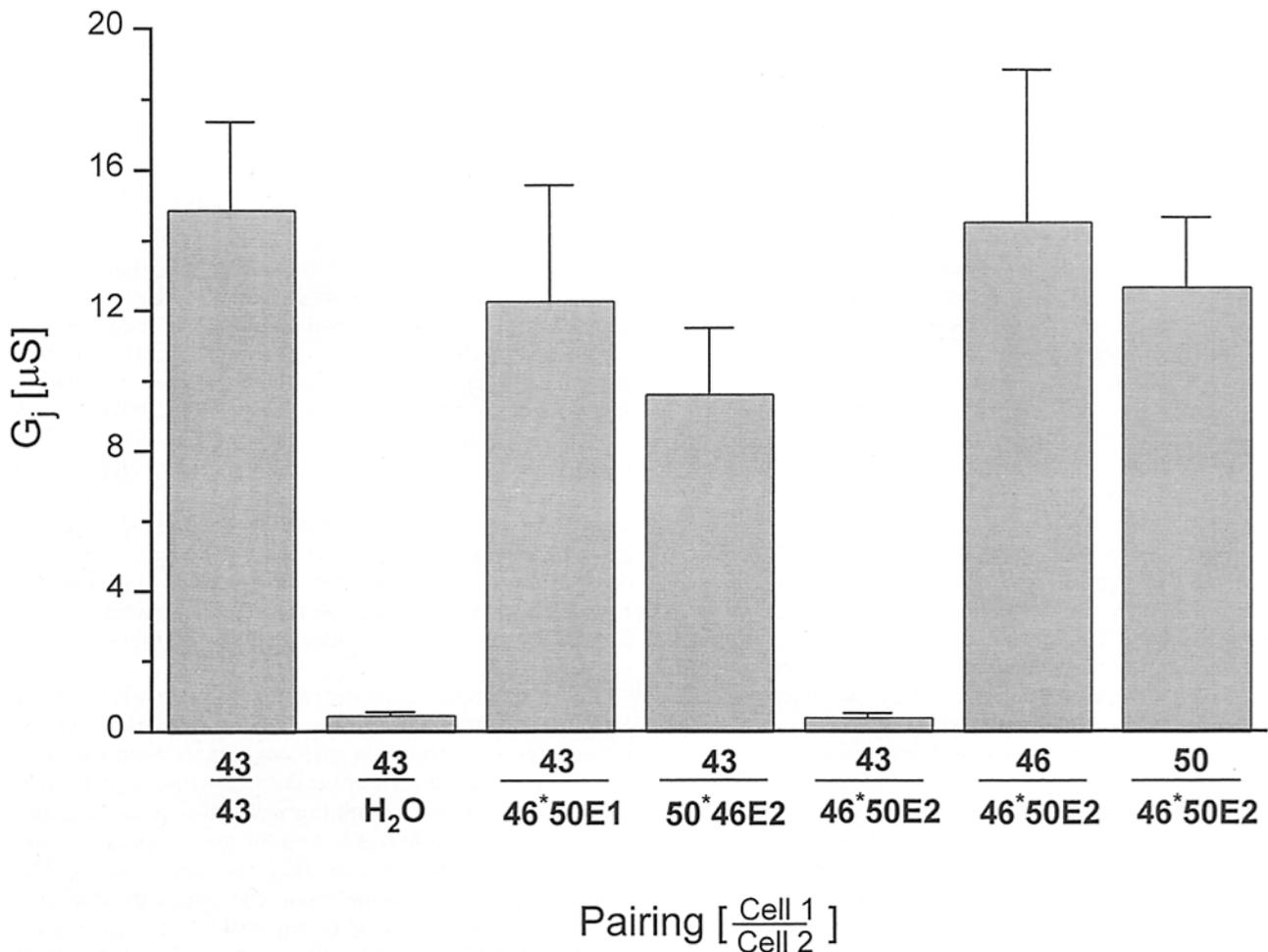


Figure 8. The second extracellular domain is a determinant of connexin compatibility. Oocytes were treated with an antisense *Xenopus* Cx38 oligonucleotide, injected with connexin mRNA or H₂O, and paired. Endogenous contributions to the conductance were <5% of the homotypic Cx43 value (43/43, $n = 28$; 43/H₂O, $n = 24$). Both chimeric Cx46*50E1 ($n = 11$) and Cx50*46E2 ($n = 17$) were able to form heterotypic intercellular channels with Cx43. Chimeric Cx46*50E2 was unable to interact with Cx43 ($n = 14$), but readily formed heterotypic channels with Cx46 ($n = 7$) or Cx50 ($n = 10$). The E1 domain of Cx50, when present in Cx46*50E1, did not prevent heterotypic communication between Cx46 and Cx43, while the E2 domain of Cx50, present in Cx46*50E2, did inhibit heterotypic interaction with Cx43, but not with Cx46 or Cx50. The E2 domain of Cx46, when present in Cx50*46E2, conferred upon Cx50 the novel ability to form heterotypic channels with Cx43.

domain is a determining factor of compatibility among different connexins.

By expressing three connexins in two cell types, the lens has the potential to generate at least five physiologically distinct classes of intercellular channels. Cx43 and Cx46 homotypic channels have voltage properties similar to those reported for Cx26, Cx30.3, and Cx32 (Barrio et al., 1991; Hennemann et al., 1992a), characterized by a high V_o and low cooperativity constant (Table III). The response of Cx43 was also slightly asymmetric because of a dependence on the potential difference between the cell cytoplasm and the outside bath, as has been reported for Cx26 (Barrio et al., 1991). Unlike Cx26, the V_{i-o} dependence of Cx43 was not apparent in the instantaneous conductance, but occurred slowly, being most evident when the decay of the junctional current was at equilibrium. Similar findings for Cx43 channels expressed in *Xenopus* oocytes have been reported in a recent abstract (Barrio et al., 1993). In contrast, Cx50

homotypic channels were very voltage sensitive, more comparable to those of Cx37 and Cx40 (Hennemann et al., 1992c; Bruzzone et al., 1993), displaying a low V_o and a very large cooperativity constant. Surprisingly, the voltage sensitivity of the heterotypic channels differed dramatically from the homotypic channels. Heterotypic channels composed of other connexins have also varied from their homotypic counterparts (Barrio et al., 1991; Hennemann et al., 1992c; Bruzzone et al., 1994), and it may be generally true that allosteric interactions between opposing connexons generate intercellular channels with novel properties.

It was also apparent that the measured steady-state conductance in heterotypic Cx46/Cx50 pairs exhibited slight deviation from the fitted Boltzmann equations at large trans-junctional potentials. For other connexins, this type of deviation has been modeled as an indication of multiple gating phenomena, which are not fit by a single Boltzmann equation (Willecke et al., 1991). As both types of homotypic channel

are well fit by the Boltzmann model, the deviation can be interpreted as a further indication that the heterotypic channels are exhibiting novel properties not observed for their homotypic counterparts.

Previous studies examining the voltage gating of Cx43 in *Xenopus* oocytes found little or no voltage sensitivity (Swenson et al., 1989; Werner et al., 1989). A major difference between our study and previous studies was the level of conductance between oocytes. In previous studies, the mean G_j was an order of magnitude greater than we have examined here, and it has been proposed that higher conductance causes an overestimation of the actual transjunctional potential because of access resistance (see Jongsma et al., 1991). Voltage sensitivity has been observed for Cx43 in primary cultures or transfected mammalian cell lines, and the Boltzmann values we obtained for Cx43 in paired *Xenopus* oocytes are in general agreement with values obtained for Cx43 expressed in other cell types (Fishman et al., 1991; Moreno et al., 1992; Spray et al., 1992).

Gap junctional conductance can also be controlled by intracellular pH (Turin and Warner, 1977; Spray et al., 1981b), and regulation by pH is independent of voltage sensitivity (Spray et al., 1986). In vivo physiological studies have previously shown that intercellular communication between chicken lens fibers was not affected by cytoplasmic acidification (Schuetze and Goodenough, 1982; Miller and Goodenough, 1986), while in rats, lens fiber intercellular communication was reported to be pH dependent (Bassnett and Duncan, 1988). The cloning of lens connexins, and their functional expression, may help to resolve this apparent discrepancy. A recently cloned chick lens fiber connexin, Cx56, has been reported to be insensitive to cytoplasmic acidification when expressed in N2A cells (Rup et al., 1993). Unlike chick Cx56, we have shown that all rodent fiber connexins exhibited pH dependence when expressed in paired oocytes. Thus, the contrasting in vivo physiology could be explained by the different molecular components that make up the lens fiber intercellular channels in this two species. We also observed that the three rodent lens connexins displayed qualitatively different responses to cytoplasmic acidification, with Cx50 showing the fastest and Cx43 showing the slowest reduction in conductance during perfusion of oocyte pairs with carbon dioxide saturated medium.

It was originally proposed that intercellular communication was an indiscriminate phenomenon, and this was supported by the observation that a wide variety of vertebrate cell types could communicate in coculture (Epstein and Gilula, 1977). Early experiments in paired oocytes support this hypothesis, demonstrating that many rodent connexins readily form heterotypic intercellular channels (Swenson et al., 1989; Werner et al., 1989; Barrio et al., 1991; Hennemann et al., 1992c). In an exception to this generalization, a recent study has demonstrated that connexins found in the cardiovascular system interact in a selective manner (Bruzzone et al., 1993). We have shown that the lens connexins form heterotypic channels only if the adjacent cell is expressing a compatible connexin. Thus selective communication may be a more general phenomenon, representing a mechanism for establishing the intercellular communication boundaries separating cellular compartments that have been observed in many systems (Lo and Gilula, 1979; Warner and Lawrence, 1982; Chanson et al., 1991).

It is interesting to speculate what role selective channel formation may play in the lens, taking into account the distribution of the three lens connexins. Connexins 46 and 50 are found in the fiber cells, and they have been colocalized in the same isolated gap junctional plaques by immunoelectron microscopy. The gap junctions joining epithelial cells to each other have been shown to contain Cx43, and neither Cx46 or Cx50 have been detected in these cells (Kistler et al., 1985; Beyer et al., 1989; Musil et al., 1990; Paul et al., 1991; White et al., 1992; Evans et al., 1993). However, the epithelial cells and the fibers are coupled to each other (Goodenough et al., 1980; Rae and Kuszak, 1983; Miller and Goodenough, 1986; but see Kuszak et al., 1991), and it is possible that the gap junction structures observed between these two cell types (Miller and Goodenough, 1986; Lo and Reese, 1993) contain heterotypic channels composed of Cx43 and Cx46. Alternatively, epithelial cells may indeed express either Cx46 or Cx50. Recently, in the embryonic chick lens, fiber connexins have been localized immunohistochemically in the epithelial cells (Jiang et al., 1994), although the adult distribution of these proteins in the chicken have not been determined. The functional importance of the lack of channel formation between Cx50 and Cx43 is currently unclear.

The extracellular domains of connexin proteins have at least two important functions: they must discriminate between alternative connexin partners, and they must be able to form an impermeant seal between the channel lumen and the extracellular space. Both extracellular domains are conserved among all connexins, with the second (E2) showing more variability than the first (E1, Haefliger et al., 1992). Each extracellular domain also contains three conserved cysteines, which were shown to be required for functional channel formation (Dahl et al., 1991, 1992). Previous studies have determined that the E2 domain is not directly involved in voltage gating, and that exchanging E2 sequences between compatible connexins does not affect their ability to form intercellular channels (Rubin et al., 1992). We have examined the molecular basis of selective communication, and demonstrated that the second extracellular domain plays a role in the determination of compatibility between the lens connexins. It is not clear which domains of the opposing connexin molecules are interacting in the extracellular space; the second extracellular domain could be interacting with E1, E2, or a combination of both domains in the opposing cell. Whether all selective connexins discriminate between different partners on the basis of their E2 sequences remains to be determined.

We are indebted to Dr. Joerg Kistler for the generous gift of monoclonal antibody 6-4-B2-C6, and to Amani Thomas-Yusuf for technical assistance. We also thank Dr. Jeff Goliger for critically reading the manuscript. These studies were supported by grants EY02430 (D. A. Goodenough) and GM37751 (D. L. Paul).

Received for publication 21 January 1994 and in revised form 15 March 1994.

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